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Antioxidative enzyme activities in human erythrocytes

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Reliable and standardized methods are necessary to determine the expression of antioxidative enzymes and their role in maintaining health. In addition, the variability of the enzyme activities within the general population caused by age, gender, and life-style factors must be described. This study describes methodological conditions that are suitable for analyzing copper–zinc superoxide dismutase (CuZn-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione reductase (GR) in human erythrocytes with a high degree of reproducibility. Intervals for the enzyme activities have been established in a randomly selected population of 220 individuals between 20 and 89 years of age. An age-related decrease was observed in CuZn-SOD and GR activities, whereas no age-related changes were demonstrated for GSH-Px and CAT. The GSH-Px activity was positively associated with the intake of dietary supplements and negatively correlated with tobacco consumption. These factors probably account for the fact that women tended to have higher GSH-Px activity.

INDEXING TERMS: superoxide dismutase • glutathione peroxidase • glutahione reductase • catalase • biological variability

Detrimental effects caused by reactive oxygen species occur as a consequence of an imbalance between the formation and inactivation of these species. Oxidative damage may be involved in the pathogenesis of major diseases such as cancer, atherosclerosis *[1]*, and certain neurological disorders *[2]*. Inactivation and removal of reactive oxygen species depend on reactions involving the antioxidative defense system. The capacity is determined by a dynamic interaction between individual components, which include vitamins A, E, and C, β-carotene, reduced glutathione (GSH), and several antioxidative enzymes.[1] The most important enzymatic antioxidants are superoxide dismutase (CuZn-SOD; EC 1.15.1.1), which catalyzes dismutation of the superoxide anion ($O_2^{\cdot -}$) into H_2O_2, which is then deactivated to H_2O by catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GSH-Px; EC 1.11.1.9). GSH-Px also reduces organic peroxides into their corresponding alcohols. GSH-Px uses GSH as a hydrogen donor whereby GSH is oxidized. The regeneration of GSH is catalyzed by glutathione reductase (GR; EC 1.6.4.2).

Wide interindividual variations may exist regarding antioxidative capacity, thus affecting individual susceptibility against deleterious oxidative reactions. However, very limited information exists concerning the biological variation of antioxidative enzymes in representative population samples. This study presents intervals for antioxidative enzyme activities from a randomly selected population. All enzyme activities except CAT were measured by automated methods.

Materials and Methods

HUMAN SUBJECTS

The sample group consisted of 220 individuals (110 women and 110 men) randomly selected from the Danish central registry of residents by using the following criteria: 20 men and 20 women in each age decade between 20 and 89 years, half of them residing within the city and the other half within rural communities. No exclusion criteria were used. Participants were included consecutively, after signing an informed consent form, until their respective subgroups were filled as described in a previous study *[3]*. The overall response and participation rates were 70% and 31% respectively. The participation rate was particularly low in the two oldest age decades (6% and 19%), and the anticipated number of subjects was not obtained in

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[1] Nonstandard abbreviations: GSH, reduced glutathione; CuZn-SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GR, glutathione reductase; DTT, dithiothreitol; Pr, protein; Hb, hemoglobin; INT, 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride; and GSSG, oxidized glutathione.

these two age decades. Information regarding dietary habits, use of dietary supplements, alcohol consumption, smoking, drug intake, working conditions, hobbies, and housing situations were obtained by interview. Approximately 60% of the sample group was currently employed. Other participants were students, retirees, or unemployed. Forty-three per cent were current smokers, 23% were ex-smokers, and 32% had never smoked. According to these data, the sample group does not seem to deviate from the general population *[4, 5]*.

To estimate within-subject variations and the effect of fasting and food intake, six healthy volunteers twice delivered blood samples three successive days and thereafter three times once a week. One week later, the six volunteers delivered a fasting blood sample. After this blood sampling a substantial breakfast rich in fat and protein was consumed by all subjects. One hour after this meal a new blood sample was drawn. To investigate the effect of different anticoagulants, venous blood samples from two volunteers were collected into Venoject tubes (VT-100 TK; Terumo, Leuven, Belgium) containing either Na-heparin or K_3-EDTA. To minimize the influence of analytical variation, all samples from each individual were analyzed in the same analytical run.

The study followed the guidelines of the Second Helsinki declaration and was approved by the Regional ethical review committee.

BLOOD SAMPLES

Venous blood samples were collected from all study subjects into Venoject tubes with EDTA (0.47 mol/L K_3-EDTA) between 1000 and 1800. All individuals were placed in a reclining position for a minimum of 10 min before blood sampling by the same phlebotomist. Within 4 h after sampling, the blood was centrifuged at 1000*g* for 10 min to separate the plasma. The buffy coat was removed and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline (9.0 g/L NaCl), and hemolyzed by adding the same weight of ice-cold demineralized ultrapure (MilliQ plus reagent grade; Millipore, Bedford, MA) water to yield a 50% hemolysate. The hemolysates were frozen in 500-μL aliquots at −80 °C for later analysis.

Erythrocytes isolated from blood samples delivered from 20 healthy blood donors were pooled and hemolyzed after the washing procedure to obtain identical aliquots to be used as control material.

ANALYTICAL METHODS

All reagents used were analytical grade. Water used was demineralized ultrapure of MilliQ plus reagent grade. All reagents except the phosphate buffers were prepared each day and stored in a refrigerator at −4 °C. The reagents were equilibrated at room temperature for 0.5 h before use when the analysis was initiated or reagent containers were refilled. Phosphate buffers were stable at −4 °C for 1 month. At the day of analysis the hemolysates were thawed and diluted 5:1 (by vol) with distilled water and further 2:1 (by vol) with a phosphate buffer containing dithiothreitol (DTT) (100 mmol/L KH_2PO_4, 1 mmol/L EDTA, 2 mmol/L DTT, pH 7.4) to a final dilution of 1:20. These 1:20 hemolysates were used for all analyses. Diluted samples not analyzed immediately were placed in a refrigerator until analysis later in the day. Protein (Pr) and hemoglobin (Hb) concentrations and all enzyme activities except CAT were determined with a Cobas Mira autoanalyzer (F. Hoffmann-La Roche, Diagnostic Systems, Basle, Switzerland). The methods were modified as stated below for the autoanalyzer procedure. To obtain optimal accuracy in pipetting, small volumes of H_2O or assay buffer were pipetted into the cuvettes together with samples and reagents to rinse the needle. These volumes are included in the final reaction volumes. All measurements were performed in triplicate.

Assay of CuZn-SOD activity. Determination of CuZn-SOD activity was performed by using a kit (Ransod; Randox Labs. cat. no. SD 125, Crumlin, UK) based on the method developed by McCord and Fridovich *[6]* coupling an $O_2^{\cdot-}$ generator with an $O_2^{\cdot-}$ detector. In the kit, xanthine and xanthine oxidase are used to generate $O_2^{\cdot-}$ and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), which reacts with $O_2^{\cdot-}$ to form a red formazan dye used as detector. CuZn-SOD inhibits the formation of the formazan dye, and the activity is measured as percent inhibition compared with a calibration curve with purified SOD. The final concentrations of the reagents used in the assay were as recommended by the manufacturer (0.05 mmol/L xanthine and 0.025 mmol/L INT in the main reagent and 80 U/L xanthine oxidase in the start reagent). The sample (1:20 hemolysate) was first diluted 10-fold in KH_2PO_4 buffer (10 mmol/L; pH 7.0) by the autoanalyzer and 5 μL (plus 20 μL of H_2O) of diluted hemolysate was added concomitantly with the main reagent (170 μL) to the cuvette. Absorbance was monitored at 500 nm for 150 s after addition of xanthine oxidase (25 μL plus 10 μL of H_2O) as start reagent. The final reaction volume was 230 μL. The unit of activity is defined as the amount of enzyme that inhibits the rate of the formazan dye formation by 50%.

Assay of GSH-Px activity. Total activity of GSH-Px was determined by the coupled enzyme procedure *[7]* with *tert*-butyl hydroperoxide as substrate. In erythrocytes from adult humans all GSH-Px activity seems to be Se dependent *[8]* and therefore the measurements represent Se-dependent GSH-Px activity. Before analysis the autoanalyzer was programmed to dilute the sample 10-fold to a 1:200 hemolysate by adding Drabkin's reagent (double strength) to inhibit the peroxidase activity of Hb. The main reagent was made by mixing 8.00 mL of KH_2PO_4 buffer (100 mmol/L; 1 mmol EDTA/L; pH 7.4), 4.00 mL of GR (5000 U/L, cat. no. G-4751; Sigma Chemical Co., St. Louis, MO), 2.00 mL of GSH (2.5 mmol/L; Sigma G-4251),

and 2.00 mL of NADPH (2.5 mmol/L; Sigma N-1630). The main reagent (200 μL) and the sample (25 μL of 1:200 hemolysate plus 10 μL of H_2O) were added to the cuvette and the absorbance at 340 nm was monitored for 200 s (step A). Then 10 μL of *tert*-butyl hydroperoxide (25 mmol/L; Sigma B-2633) (plus 5 μL of H_2O) were added as start reagent. The absorbance was monitored for another 225 s (step B). The final reaction volume was 250 μL. The difference in absorbance per minute between steps B and A was used to calculate the enzyme activity by using a molar absorptivity of NADPH at 6.22×10^3 L mol^{-1} cm^{-1}. The unit is μmol of NADPH oxidized/min.

Assay of GR activity. Activity was determined by following the oxidation of NADPH to $NADP^+$ during the reduction of oxidized glutathione (GSSG) *[9]*. The main reagent was prepared by combining 18.00 mL of KH_2PO_4 buffer (139 mmol/L, 0.76 mmol/L EDTA; pH 7.4) and 2.00 mL of NADPH (2.5 mmol/L). The sample (20 μL of 1:20 hemolysate plus 20 μL of KH_2PO_4 buffer), 220 μL of the main reagent, and 5 μL of FAD (315 μmol/L) plus 10 μL of KH_2PO_4 buffer were added to the cuvette, and the absorbance at 340 nm was monitored for 200 s (step A). Then 30 μL of GSSG (22 mmol/L; Sigma G-4376) plus 10 μL of KH_2PO_4 buffer were added to start the reaction and the absorbance was followed for 175 s. The final reaction volume was 315 μL. The difference in absorbance per minute between steps B and A was used to calculate the enzyme activity by using a molar absorptivity of NADPH at 6.22×10^3 L mol^{-1} cm^{-1}. The unit is μmol of NADPH oxidized/min.

Assay of CAT activity. CAT activity was determined by the method described by Aebi *[10]* by using a UV/visible spectrophotometer (lambda 11; Perkin-Elmer, Norwalk, CT). The decomposition rate of the substrate H_2O_2 was monitored at 240 nm. A molar absorptivity of 43.6 L mol^{-1} cm^{-1} was used to calculate the activity. One unit is equal to 1 μmol of H_2O_2 decomposed/min.

Hb and Pr concentrations. Hb and Pr concentrations in the 1:20 hemolysates were determined spectrophotometrically on the Cobas Mira by standard kits. The Hb assay (Sigma cat. no. 525-A) is based on the colorimetric cyanomethemoglobin method, and the Pr assay (Sigma cat. no. 541–2) is based on the colorimetric biuret method. The specific enzyme activities were expressed as U/g Hb or U/g Pr.

Quality assurance. For all enzymes, identical samples of control material were analyzed repeatedly during each day to adjust for analytical day-to-day variations.

STATISTICS

Statistical analyses were performed by using the SPSS 6.0 software (SPSS, Chicago, IL). Goodness of fit to normal distributions were investigated by probit plots and the Lilliefors test. GSH-Px and CAT values showed normal distributions. The logarithmic transformation of CuZn-SOD and GR results also approached the normal distribution. Multiple regression analysis with backward elimination was used to investigate the influence of different variables on the enzyme activities. The criterion for removing variables from the model was chosen at $P > 0.10$. Spearman's rank correlation coefficient was used to investigate associations between enzyme activities. Differences in means between groups were analyzed by the independent-samples *t*-test. The effects of fasting and eating on enzyme activities were analyzed by paired *t*-test. Intervals for the enzyme activities were established by using the program REFVAL 3.42. This program implements the methods for statistical treatment of reference values recommended by the IFCC. Two-tailed *P* values are given throughout.

Results

The precision of the analytical methods was assessed by repeated assays of pools of hemolysates. The CVs for within-day repeatability and day-to-day reproducibility are shown in Table 1. Within-run CVs express the precision of the autoanalyzer. Since CAT activity was measured by a manual assay, no within-run CV for CAT is available. The within-run CVs obtained for the enzyme analysis on the autoanalyzer by measuring 10 consecutive aliquots of a sample were always <3%. The within-day variations ranged between 2.1% and 6.5% for the various enzyme analyses. The highest day-to-day variations were seen for CuZn-SOD and GSH-Px. To minimize the influence of analytical variations, the values measured were adjusted by using the control samples as internal standards. Thus the day-to-day variations for CuZn-SOD, GSH-Px, and GR were reduced considerably (Table 1). Within-day CVs for Hb and Pr determinations were <1% and <2% and day-to-day CVs <3.5% and <2.5% respectively. No storage-related changes in mean enzyme activities were observed for up to 6 months at −80 °C. The enzyme activities in the control hemolysates were therefore considered stable.

The activities of the four antioxidative enzymes obtained in this study are shown in Table 2. The results of the statistical analysis were independent of whether the enzyme activities were expressed per gram of Pr or gram of Hb. All enzyme activities were independent of whether the study person was fasting or not and whether the blood samples were collected into tubes containing Na-heparin or K_3-EDTA as anticoagulant.

The within-subject variations are shown in Table 3. The between-subject variations for the 220 subjects in the sample group were 11.1% for CuZn-SOD, 16.6% for GSH-Px, 12.3% for GR, and 9.4% for CAT. Thus, the mean within-subject variation is approximately half as large for all of these enzymes. The highest within-subject variations for CuZn-SOD and GSH-Px were almost as large as the between-subject variation.

Table 1. CVs for antioxidative enzyme measurements in erythrocytes.

	CuZn-SOD, U/L	CAT, kU/L	GSH-Px, U/L	GR, U/L
Within-run				
n	10		10	10
Mean	8531		1545.6	146.3
CV, %	2.7		3.0	2.1
Within-day				
n	16	12	12	12
Mean	10683	2179.6	1712.6	142.0
CV, %	6.5	2.8	4.9	2.1
Day-to-day				
n	12	7	11	11
Mean	11314	2204.2	1693.9	141.7
CV, %	5.6	3.7	5.7	2.8
Mean[a]	11384	2085.3	1570.9	123.2
CV, %[a]	4.3	4.0	4.8	1.6

[a] After adjustment for analytical day-to-day variations by including identical samples of control material in each analytical run.

The covariates included in the regression model were age (years), gender (0 = men, 1 = women), smoking (0 = no, 1 = yes), tobacco consumption (g/day), alcohol (drinks/week), caffeine intake (cups of coffee/day; 1 cup of tea = ½ cup of coffee), and dietary supplements (0 = no, 1 = yes).

Use of drugs within the last month was reported by 83 individuals. Of these, 32 had used more than one drug. The drugs were classified according to WHO *[11]* and the most frequently used types of drugs were sex hormones including oral contraceptives (n = 28), analgesics (n = 26), nonsteroidal antiinflammatory drugs (n = 12), diuretics (n = 11), psycholeptics (n = 9), antiasthmatics (n = 8), antibacterials (n = 6), and calcium channel blockers (n = 5). Other drug types were used by less than five individuals. Drug treatment was not included in the regression model since most drug types were only used by a few individuals. In addition, it is not possible in this study to separate effects from drug treatment from effects of the disease on enzyme activities. When means of enzyme activities were compared between groups using specific drugs types and individuals using no drugs, a tendency towards higher GR activity was observed among users of analgesics ($P = 0.06$). Only groups consisting of 10 or more individuals were analyzed. No other drug-related effects on enzyme activities were observed.

Table 2. Enzyme activities of CuZn-SOD, GSH-Px, GR, and CAT in erythrocytes for 220 individuals (both sexes) between 20 and 89 years of age.

Enzyme	0.025 Fractile (0.90 confidence interval)	0.975 Fractile (0.90 confidence interval)
CuZn-SOD		
U/g Pr	418 (408–429)	655 (637–674)
U/g Hb	748 (732–764)	1194 (1162–1226)
GSH-Px		
U/g Pr	47.08 (45.21–48.97)	91.68 (88.98–94.47)
U/g Hb	86.32 (83.13–89.55)	165.55 (160.47–170.84)
GR		
U/g Pr	3.48 (3.35–3.61)	5.81 (5.63–6.00)
U/g Hb	6.30 (6.08–6.52)	10.33 (10.05–10.64)
CAT		
kU/g Pr	73.14 (71.78–74.53)	106.01 (104.08–108.00)
kU/g Hb	130.36 (127.76–132.99)	192.27 (188.52–196.13)

The activity of CuZn-SOD significantly decreased with age (Table 4, Fig. 1). None of the other variables recorded had any significant effect on the CuZn-SOD activity.

In contrast, no age relation was found for GSH-Px. Daily intake of dietary supplements was associated with an increased activity of GSH-Px, whereas the amount of tobacco consumption was negatively correlated with GSH-Px activity (Table 4). GSH-Px activity was slightly higher in women than in men (Table 4; Fig. 1). Women used dietary supplements more frequently than men (43.6% and 27.5% respectively); therefore the effects of dietary supplements and gender on GSH-Px activity are difficult to separate. However, the tolerance was high (0.97) and variance inflation factor low (1.03) for gender in the final regression model, indicating separate influence on the variance from the two variables. The mean activity of GSH-Px was significantly lower in current smokers than in nonsmokers ($P = 0.028$). However, some of this difference may be explained by a tendency toward fewer users of dietary supplements among smokers and a higher level of tobacco consumption among men than women (data not shown). Thus, according to the regression analysis, smoking was not significantly associated with GSH-Px activity when adjustment for other variables was included (Table 4).

The activity of GR decreased slightly with age (Table 4; Fig. 1) and with smoking.

The activity of CAT was not appreciably influenced by any of these variables. The CAT activity as a function of age for both sexes is shown in Fig. 1. Alcohol and caffeine intake were not associated with any of the enzyme activities.

The activity of CuZn-SOD was positively correlated to CAT ($r = 0.26$; $P < 0.0001$) and GR ($r = 0.16$; $P = 0.02$), and CAT was positively correlated to GR ($r = 0.21$; $P = 0.002$). No significant correlations were found between GSH-Px and any of the other enzymes measured.

Discussion

Antioxidative enzymes may play an important role in determining individual risk of developing certain diseases, such as cancer or atherosclerosis. Besides constitutional individual differences in gene expression, antioxidative enzyme activities apparently depend on variations in life-style and environmental factors, since within-subject variations are greater than the analytical imprecision. The relatively high analytical variations of CuZn-SOD and GSH-Px is probably caused by unavoidable differences in manual weighing and pipetting of reagents as

Table 3. Within-subject variations of CuZn-SOD, GSH-Px, GR, and CAT activities in erythrocytes in six healthy volunteers.[a]

Subject no.	CuZn-SOD, U/g Pr		GSH-Px, U/g Pr		GR, U/g Pr		CAT, kU/g Pr	
	Mean	CV, %	Mean	CV, %	Mean	CV, %	Mean	CV, %
1	372	3.8	52.8	4.4	4.5	4.0	74.5	2.9
2	470	7.5	78.6	4.4	4.5	3.5	87.4	5.7
3	446	6.4	46.4	4.9	4.0	3.5	71.6	4.7
4	420	6.6	43.8	5.3	4.8	7.0	71.9	5.1
5	434	4.9	46.3	10.6	3.6	6.9	67.2	4.7
6	386	9.4	35.5	13.0	3.8	5.9	66.5	4.6

[a] Nine blood samples from each individual delivered twice three successive days and thereafter three times once a week.

well as small variations between kits. The methods used for measuring CAT and GR are less sensitive to small variations in reagent concentrations, since the reagents added in these assays are not rate limiting.

Antioxidative enzyme activities vary among different tissues, and environmental factors might affect the enzyme activities only in susceptible organs. Therefore, the activities found in erythrocytes do not necessarily reflect the antioxidative defense of the whole organism. In this study, variations between individuals are generally substantially larger than within-subject variations, thus suggesting that the enzyme activity in a blood sample may reflect differences in the antioxidative defense.

Most previous studies were designed to compare activities of antioxidative enzymes in groups of patients with certain diseases with matched controls. Observed differences in enzyme activities in most of these studies could constitute a contributory cause of the disease, a result of the disease, or an effect of drug treatment. In addition, analytical differences between laboratories make it difficult to compare the results obtained in different studies. Although most laboratories use methods based on the same methodological principles, the specific assay conditions regarding concentration of reagents, recording time, and temperature differ.

A small number of other studies have addressed the biological variability in antioxidative enzymes *[12–14]*. However, these studies are apparently not based on randomly selected population groups. In the present study, an age-related decrease was observed in CuZn-SOD and GR activities in erythrocytes. No obvious age-related changes were observed for CAT or GSH-Px, although GSH-Px tended to increase slightly with age. These results are in accordance with a French study *[12]* based on 167 individuals between 1 month and 67 years of age; a negative correlation between age and activities of CuZn-SOD and GR and a positive correlation between age and GSH-Px activity in erythrocytes were reported. In another French study *[13]* involving 1782 individuals between 4 and 65 years of age and 54 subjects between 65 and 97 years, the activities of CuZn-SOD, CAT, and GSH-Px in erythrocytes decreased with age, especially in individuals >65 years of age. GR was not investigated. An age-related decrease in CuZn-SOD activity in erythrocytes was also reported from a Polish population (n = 93) between 4 and 80 years of age, and the activities of CAT and GSH-Px increased with age *[14]*. Hence, an age-related decrease in CuZn-SOD activity in human erythrocytes seems to be a rather consistent finding, whereas some discrepancy exists regarding the age relation of the other antioxidative enzymes. Some differences between the studies may be due to factors other than age, since random population samples were not used, and selection bias may have affected the results.

Of the enzymes investigated, GSH-Px seems to be most clearly influenced by life-style and environmental factors, as indicated in this study by intake of dietary supplements and smoking habits. In other studies, smoking

Table 4. Multiple regression analysis of antioxidative enzyme activities in erythrocytes and potential determinant variables.

Variables	ln CuZn-SOD, U/g Pr		GSH-Px, U/g Pr		ln GR, U/g Pr	
	B (SE)[a]	P	B (SE)	P	B (SE)	P
Age	−0.001 (0.0005)	0.015	—		−0.001 (0.0005)	0.066
Gender	—		2.55 (1.51)	0.092	—	
Tobacco consumption	—		−0.21 (0.02)	0.025	—	
Smoking	—		—		−0.03 (0.02)	0.075
Dietary supplements	—		3.69 (1.58)	0.020	—	
Intercept	6.31		66.56		1.57	
Adjusted R^2	0.023		0.061		0.023	
R SE	0.108		10.85		0.122	

[a] Regression coefficient (standard error).

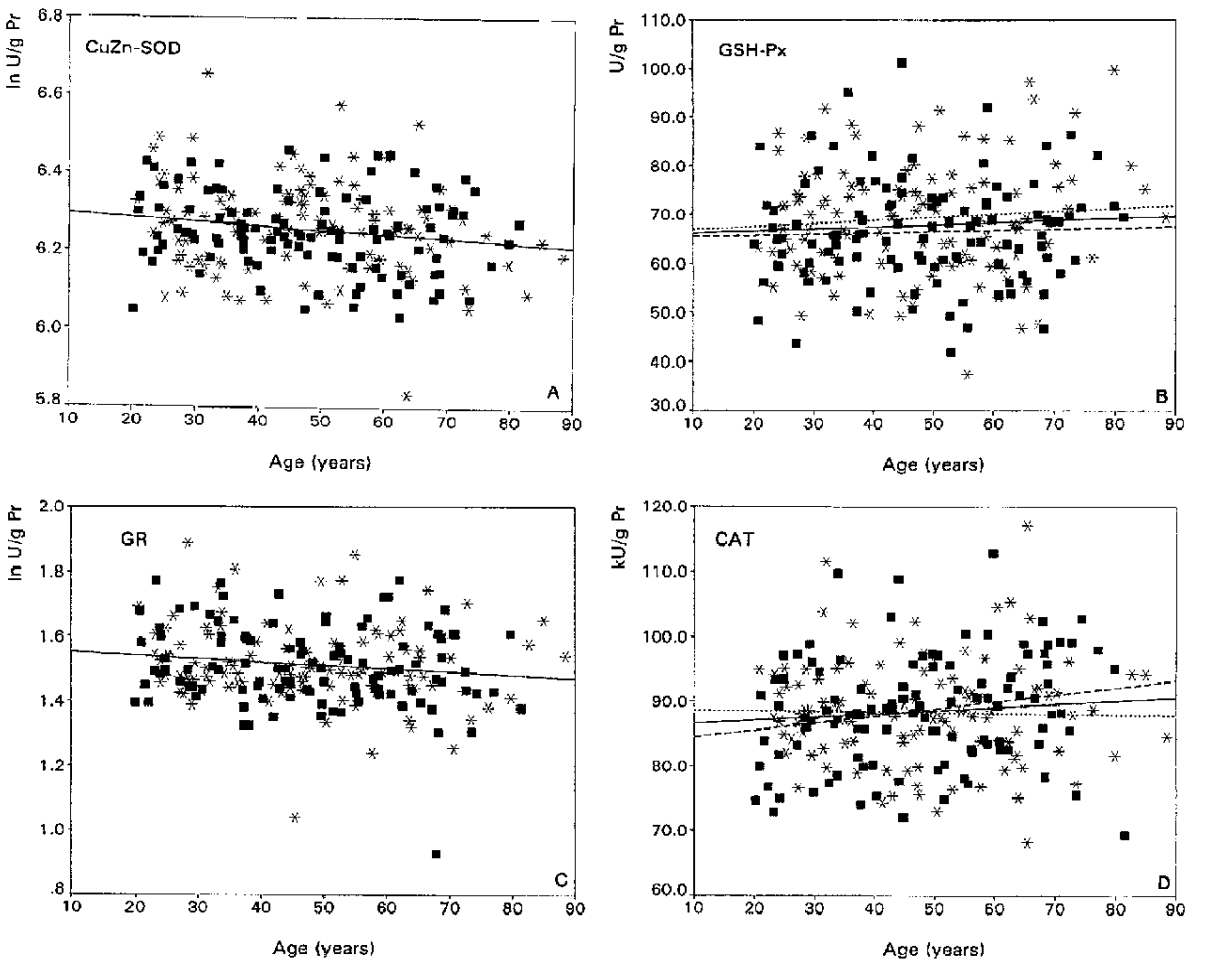

Fig.1. Scatterplot of ln CuZn-SOD (*A*), GSH-Px (*B*), ln GR (*C*), and CAT (*D*) activities against age.
■, (- - -), men; ✳, (. . . .), women; (————), total population.

habits did not influence GSH-Px activity in erythrocytes *[13, 15]*, but a decreased activity of GSH-Px in plasma has been observed in smokers *[16]*. The higher GSH-Px activity among users of dietary supplements is probably due to higher Se intake from supplements containing minerals *[17]*. A significantly higher GSH-Px activity in women than in men has been found in some studies *[13, 18]* but not in others *[12]*. In the present study, women tended to have higher GSH-Px activity, but the small difference could be due to chance or to gender-related differences in life-style such as intake of dietary supplements.

Measurement of antioxidative vitamins and other essential nutrients in human blood samples has been recommended to assess the optimal intake *[19]*. However, the application of measurements of antioxidative factors has yet to be elucidated in greater detail. First, reliable methods to measure antioxidative enzymes as well as other antioxidants in human blood samples have to be established and standardized. In addition, the variation in antioxidant concentrations in the general population and the influence of age, gender, life-style, and dietary and environmental factors on these parameters have to be estimated before the role of antioxidants in disease prevention can be further investigated.

In conclusion, this study describes measurement conditions that are suitable and reliable for analyzing antioxidative enzymes in human erythrocytes with a high degree of reproducibility. The advantages of using automated assays include reduced analytical variation, considerable reductions in time, and smaller sample volumes, when compared with manual assays. Intervals for antioxidative enzyme activities in erythrocytes from a randomly selected population have been established. These results can be used in the process of defining reference intervals for the antioxidative enzymes. Depending on the intended

use of these reference intervals, the influence of age, gender, state of health, and life-style factors on the enzyme activities should be considered.

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